Analysis of Components, Contaminants, and Impurities in Fungicide Formulations by GC/MS and LC/MS

Application

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Author

James D. McCurry
Paul Zavitsanos
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808-1610
USA

Abstract

A commercially available fungicide formulation was analyzed by both gas chromatography/mass spectrometry (GC/MS) and electrospray ionization liquid chromatography/mass spectrometry (ESI-LC/MS). The GC/MS analysis provided a detailed look at the volatile components in the formulation, but did not yield any results for the active ingredient, triforine. The ESI-LC/MS provided information on the stereoisomers of triforine as well as the nonvolatile surfactants and contaminants in the formulation. This paper demonstrates the complementary nature of these two analytical techniques when trying to fully characterize a complex chemical formulation containing a broad range of components.

Introduction

Gas chromatography/mass spectrometry (GC/MS) is an indispensable tool for solving complex problems in the chemical industry. This fast and powerful technique yields detailed information about the expected compounds in the mixture along with any unexpected impurities and breakdown products that can affect product quality. However GC/MS can only provide meaningful information for compounds that are volatile, nonionic, thermally stable, and have relatively low molecular weight. Liquid chromatography is much better suited to analyzing compounds that are nonvolatile, ionic, polar, thermally labile, or have high molecular weight. This includes about 80% of all known organic compounds [1]. When coupled with a modern atmospheric pressure ionization (API) mass spectrometer, LC/MS offers a complementary tool to GC/MS in the chemical diagnostic laboratory.

Commercial pest control formulations contain one or more active compounds along with a recipe of ingredients that can play an important role in the product's efficacy. These "inactive" ingredients are often a combination of solvents and surfactants that allow for easy application and dispersal of the active ingredient onto the target substrate. For this work, an over-the-counter fungicide formulation was purchased at a local home products store. The active ingredient in this product is 6.5 % (wt) of N,N-[1,4-piperazinediylbis(2,2,2-trichloroethylidene)] bisformamide. This is also known as triforine (CAS registry number 26644-46-2), and the structure is shown in Figure 1. The "inactive" ingredients in this formulation are listed as cyclohexanone, N-methyl pyrrolidone, and Atlox 3406-F. The Atlox 3406-F is an agricultural dispersant that contains ionic and nonionic surfactants and mixed aromatic solvents.

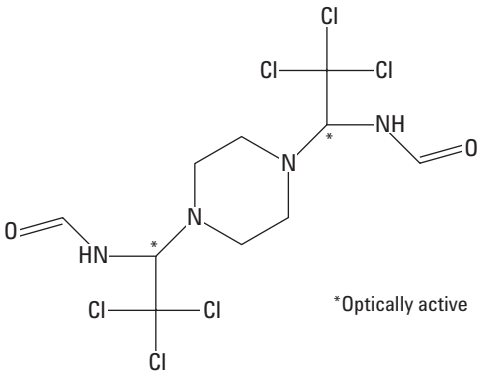

Figure 1. Chemical structure of triforine, the active ingredient in some commercial fungicides. The nominal molecular weight is 432, and the structure contains two optically active carbons.

A complete analysis of this formulation requires GC/MS to separate and identify the volatile components and LC/MS for the surfactants and polar components. Analysis of the active ingredient, triforine, presents a separate challenge. References for triforine analysis cite gas chromatography as the method of choice when analyzing environmental residues [2]. However, the melting point is reported to be 155 °C with decomposition, indicating that gas chromatography may only be possible with on-column injection.

Experimental

Gas Chromatography/Mass Spectrometry (GC/MS)

A 1% (v/v) solution of the triforine formulation was made in acetonitrile and the GC/MS analysis was performed with an Agilent 5973 GC/MS system. The components in this system were a 6890N gas chromatograph, a 7683 autoinjector, and a 5973 mass spectrometer. A cool-on-column inlet in the Agilent 6890 GC was used to avoid decomposition of the triforine. Instrument conditions for the GC/MS analysis are listed in Table 1.

Table I. GC/MS Analysis Conditions

Gas chromatograph conditions	
Column:	30 m × 0.25 mm HP5-MS, 0.25 µm (p/n 19091S-433)
Carrier gas:	Helium at 13.00 psi
Flow rate:	1.6 mL/min., constant flow mode
Inlet:	Cool on-column at 50 °C, oven track mode
Oven temperature program:	50 °C for 3 min 10 °C/min to 275 °C 275 °C for 4 min
MS Transfer line:	280 °C
Injection volume:	1 µL
Mass spectrometer conditions	
Electron multiplier:	1400 V
Solvent delay:	3 min
Scan range:	30 to 800 *m/z*
Scan threshold:	50 counts
A/D Samples:	2
Scan rate	1.95 scans/s

Electrospray Ionization Liquid Chromatography/Mass Spectrometry (ESI-LC/MS)

The same fungicide sample was run on the Agilent 1100 Series LC/MSD. This system included a vacuum degasser, a binary pump, an autoinjector, a thermostatted column compartment, and the LC/MSD SL quadrupole mass spectrometer. LC/MS instrument conditions for this analysis are shown in Table 2.

Results and Discussion

Gas Chromatography/Mass Spectrometry (GC/MS)

The complex nature of this fungicide formulation is revealed when one looks at the GC/MS data. Figure 2 shows the total ion chromatogram (TIC) of the fungicide sample. The volatile components

Table 2. LC/MS Analysis Conditions

Liquid chromatograph conditions	
Column:	150 × 4.6 mm Zorbax® XDB-C8, 5 µm (p/n 993967-906)
Mobile phase A:	0.1% Formic acid in water
Mobile phase B:	0.1% Formic acid in acetonitrile
Mobile phase gradient:	30% B at 0 min; 50% B at 7 min; 95% B at 10 min
Flow rate:	1.0 mL/min
Column temperature:	30 °C
Injection volume:	1 µL
Mass spectrometer conditions	
Source:	Electrospray
Drying gas flow:	12 L/min
Nebulizer:	40 psig
Drying gas temperature:	350 °C
V_{cap}:	3500 V (positive) and 3000 V (negative)
Stepsize:	0.1 amu
Peak width:	0.1 min
Time filter:	On
Scan range	120 to 1200 *m/z*
Fragmentor	Fixed at 60 V

in the formulation are easily identified from the mass spectral data. The major solvents, cyclohexanone and N-methyl-2-pyrrolidone, dominate the chromatogram while smaller amounts of C9 aromatics, C10 aromatics, and substituted napthalenes are easily separated and identified.

There were no peaks in the TIC whose spectra matched the triforine reference spectra from the Wiley mass spectral library. An extracted ion profile using the triforine base peak of 203 *m/z* did not produce any chromatographic peak indicating the presence of triforine. From this data, it appears that the triforine did not elute from the column into the mass spectrometer. However, a spectral average of the large hump between 18 and 20 minutes shows an isotope pattern indicating one chlorine atom (Figure 3A). Since no chlorine-containing species other than triforine are components in the formulation, the presence of chlorine and the broad peak shape indicates triforine decomposition in the gas chromatograph. The peak at approximately 20-minute retention time also has a mass spectrum containing an isotope pattern indicating the presence of two chlorine atoms in the structure (Figure 3B). This peak could be a decomposition product or a contaminant in the formulation.

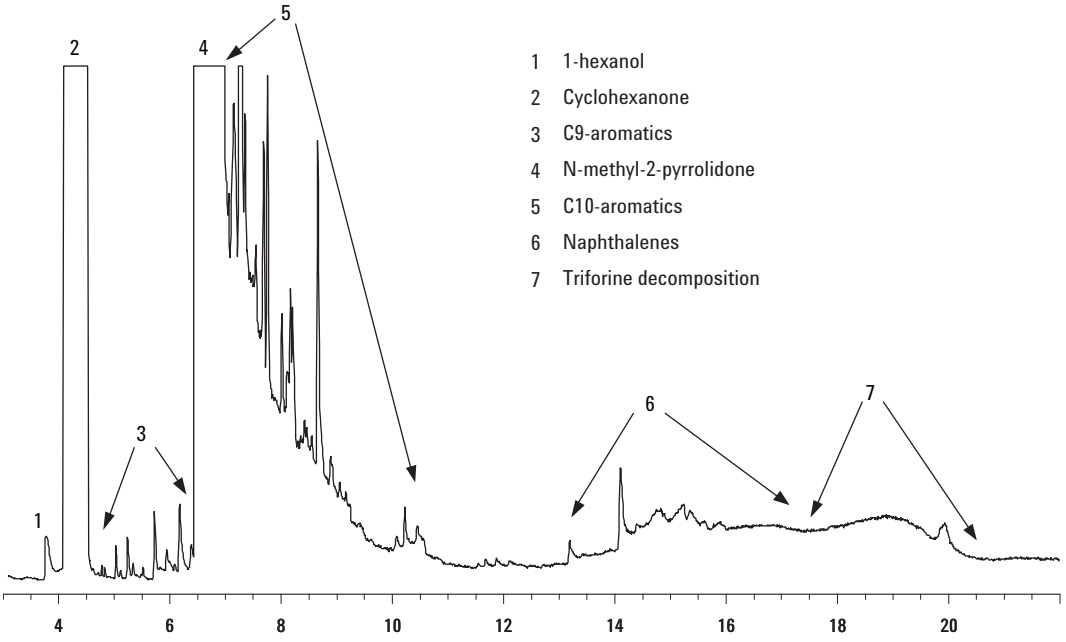

Figure 2. GC/MS TIC showing the complex volatile components in the commercial fungicide formulation.

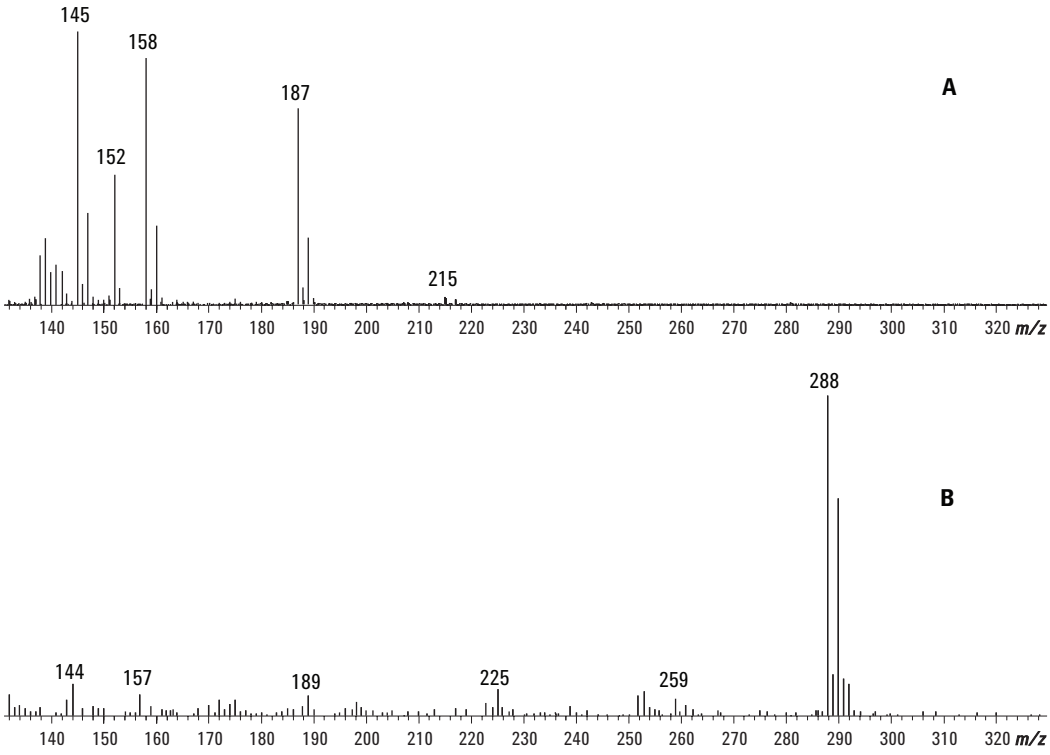

Figure 3. (A) Average mass spectrum of broad hump between 18 and 20 minutes of TIC. Isotope patterns of the peaks at *m/z* 145, 158 and 187 indicate the presence of one chlorine atom. (B) Mass spectrum of the peak at 20 minutes shows the presence of two chlorine atoms in the structure.

Electrospray Ionization Liquid Chromatography/Mass Spectrometry (ESI-LC/MS)

The positive ion ESI-LC/MS chromatogram is shown in Figure 4. Several major peaks are observed along with several minor components. The spectra of the three peaks eluting between 0 and 2 minutes are shown in Figure 5. Since electrospray is a "soft" ionization technique, these spectra do not exhibit the detailed fragmentation needed to interpret structures for these three compounds. However, peak number 2 does have an isotopic pattern indicating the presence of two chlorine atoms in the structure. This compound could be a contaminant related to triforine production or a triforine decomposition product. Figure 6 shows the spectra of the three peaks between 10.5 and 13 minutes. These compounds are the various surfactants that make up the agricultural dispersant used in the formulation.

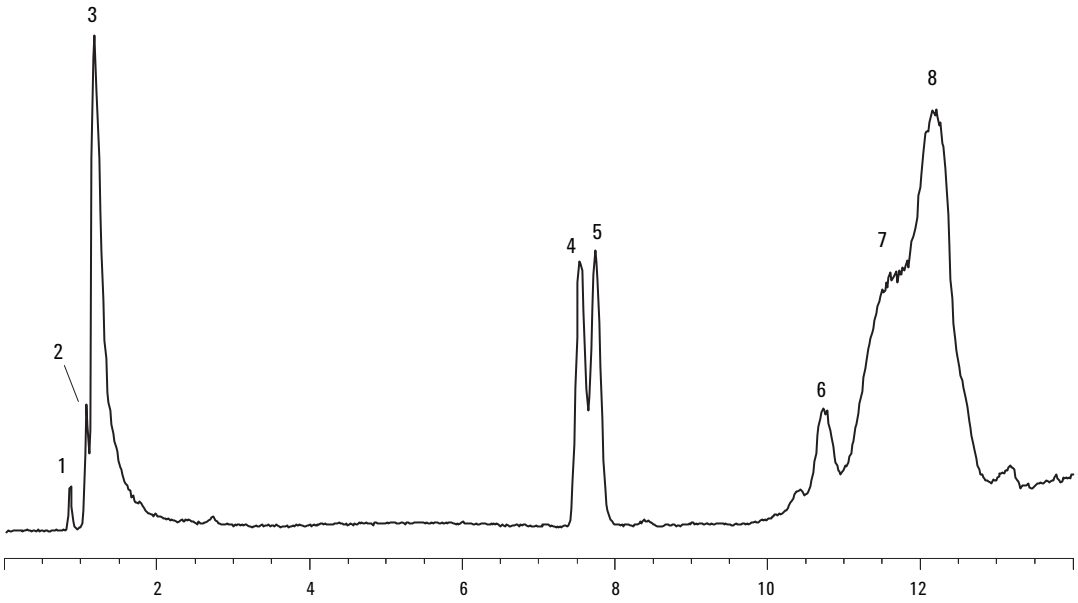

Figure 4. TIC from positive ion ESI-LC/MS of fungicide formulation.

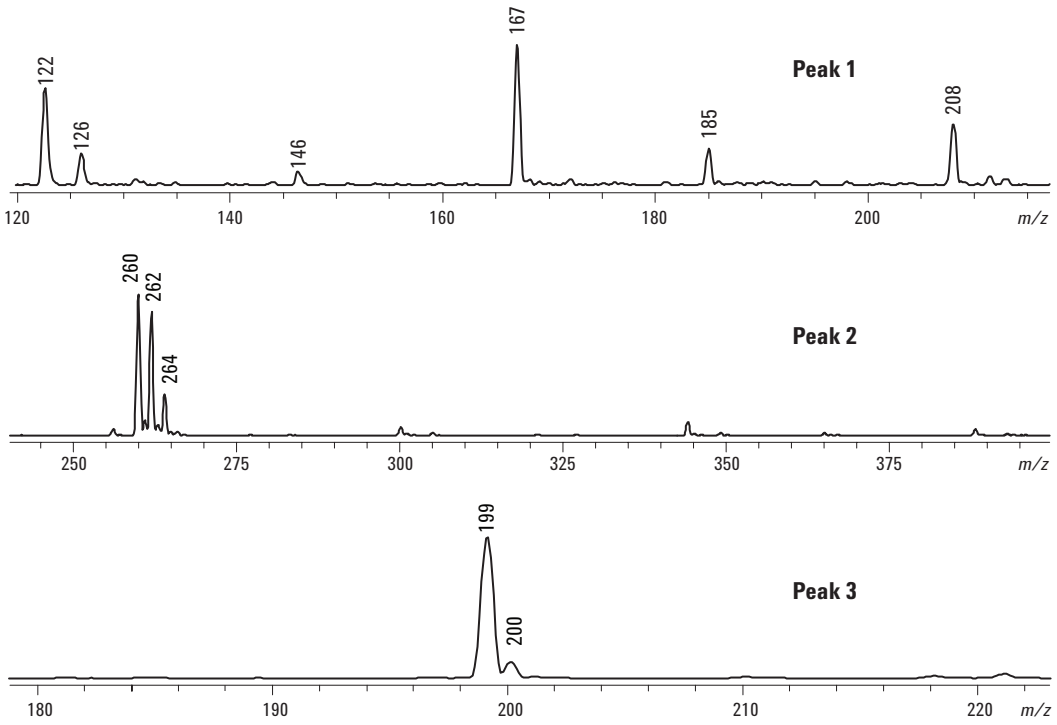

Figure 5. Electrospray spectra from LC/MS peaks 1, 2, and 3. The spectra from peak 2 shows an isotope pattern indicating two chlorine atoms in this structure. This compound may be a contaminant in the formulation from the active ingredient triforine.

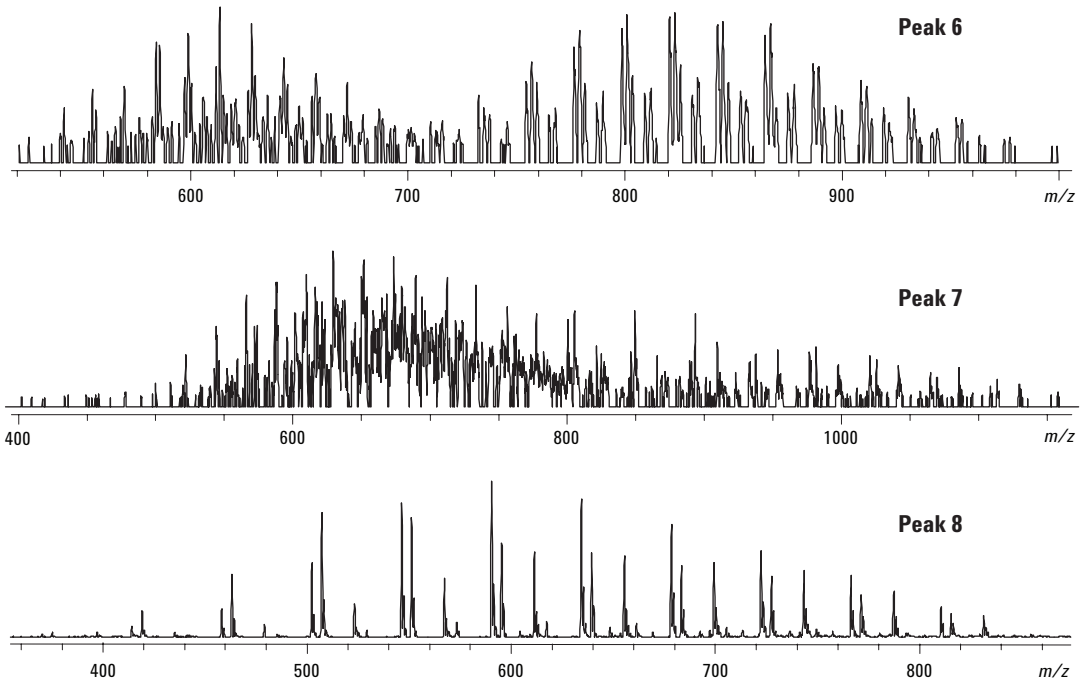

Figure 6. Electrospray mass spectra of LC/MS peaks 6, 7, and 8 from Figure 4. These compounds are the surfactants used in the formulation.

The spectra of LC/MS peaks 4 and 5 (Figure 7) are identical and correspond to the active ingredient, triforine. The protonated molecular ion is observed at *m/z* 433 along a sodium adduct at *m/z* 455. The multiplets for *m/z* 433 to 439 and *m/z* 455 to 461 exhibit an isotopic pattern consistent with six chlorine atoms. The ion at *m/z* 388 is due to a rearrangement and subsequent loss of a formamide group from the protonated molecular ion (*m/z* 433). This is also confirmed by the isotopic pattern indicating six chlorine atoms (*m/z* 388 to 396).

The presence of two triforine peaks in Figure 4 can be explained by the stereochemistry of the structure. Triforine contains two optically active carbons that give rise to four stereoisomers. Figure 8 shows the four configurations that can be grouped into two pairs of mirror images that are diastereomers. The S,R and R,S configurations are mirror images that are superimposable, resulting in a meso compound that exhibits no optical activity or differences in physical properties. Therefore, because the S,R and R,S configurations are identical, they will elute as one chromatographic peak. The second pair of mirror images are the R,R and S,S configurations. These are not superimposable and are, therefore, enatiomers that will exhibit different optical activity, but identical physical properties. Conventional reverse-phase liquid chromatography cannot separate these enantiomers, and they will co-elute as a single peak. However, these enantiomers are not mirror images of the meso compound and can be chromatographically separated from the meso compound. This is why there are two triforine peaks, one for the meso compound and one for the enatiomers. Without pure standards of the stereoisomers, it is not possible to determine which configurations can be attributed to the observed chromatographic peaks.

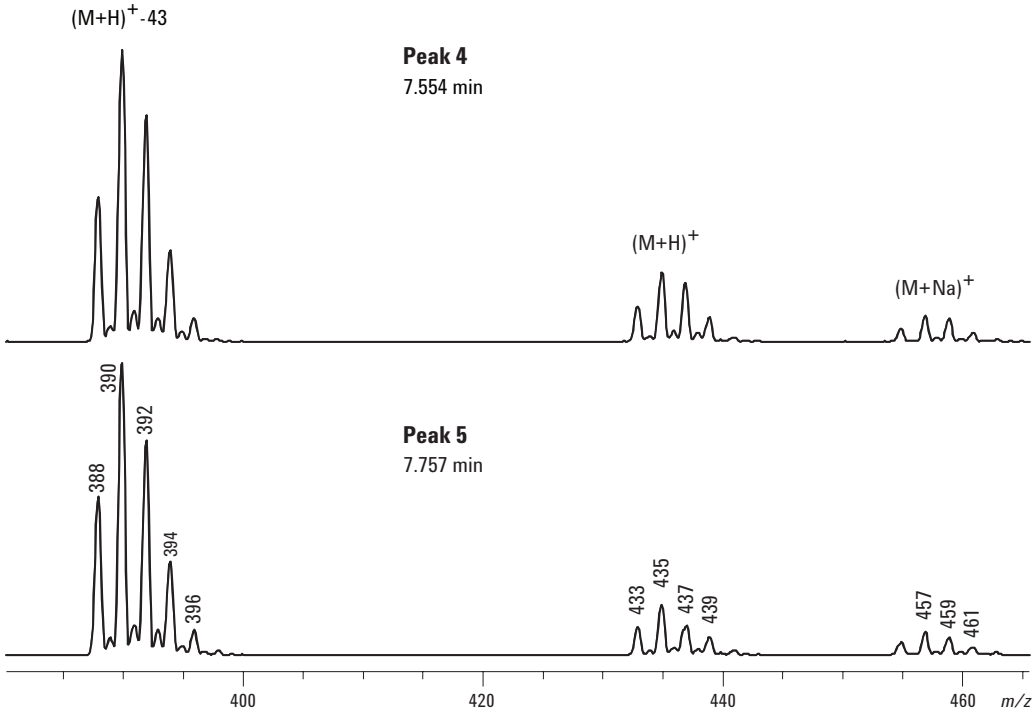

Figure 7. Electrospray mass spectra of peaks 4 and 5 from Figure 4. Both spectra show a protonated molecular ion at *m/z* 433 representing the active ingredient triforine. There is also a sodium adduct (*m/z* 455) of triforine observed for both peaks. A rearrangement and loss of a formamide group from the protonated molecular ions give rise to the multiplet at *m/z* 388 to 396.

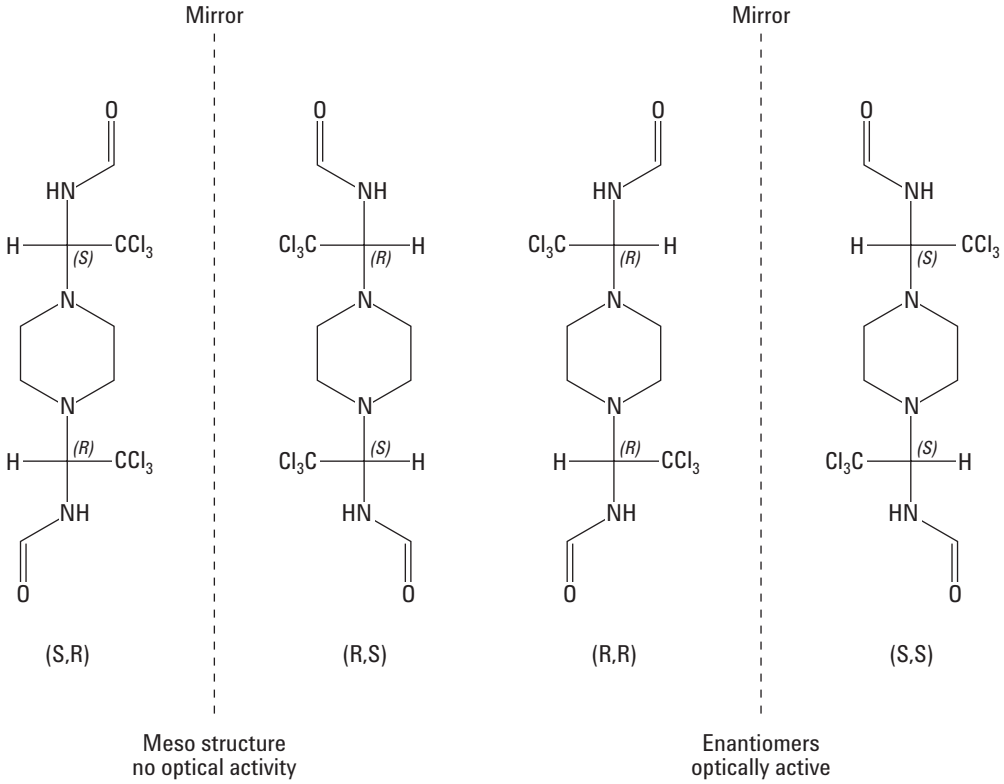

Figure 8. The four triforine stereoisomers arising from the two chiral carbons in the structure. These two pairs of mirror images account for the two triforine peaks observed in the chromatogram (Figure 4).

The fungicide formulation was also run by ESI-LC/MS in the negative ion mode. The results of this analysis are shown in Figure 9. The negative ion mass spectra for these two peaks are shown in Figure 10. For both triforine peaks, the most stable negative ion species is the chloride adduct (*m/z* 467). However, the spectra for the first peak contains a deprotonated molecular ion (*m/z* 431) and a formate adduct (*m/z* 477) that is not observed in the spectra of the later eluting peak. This selective adduct formation is likely related to the stereochemstry of the triforine, but again, without pure standards, the correct configurations cannot be assigned to the chromatographic peaks.

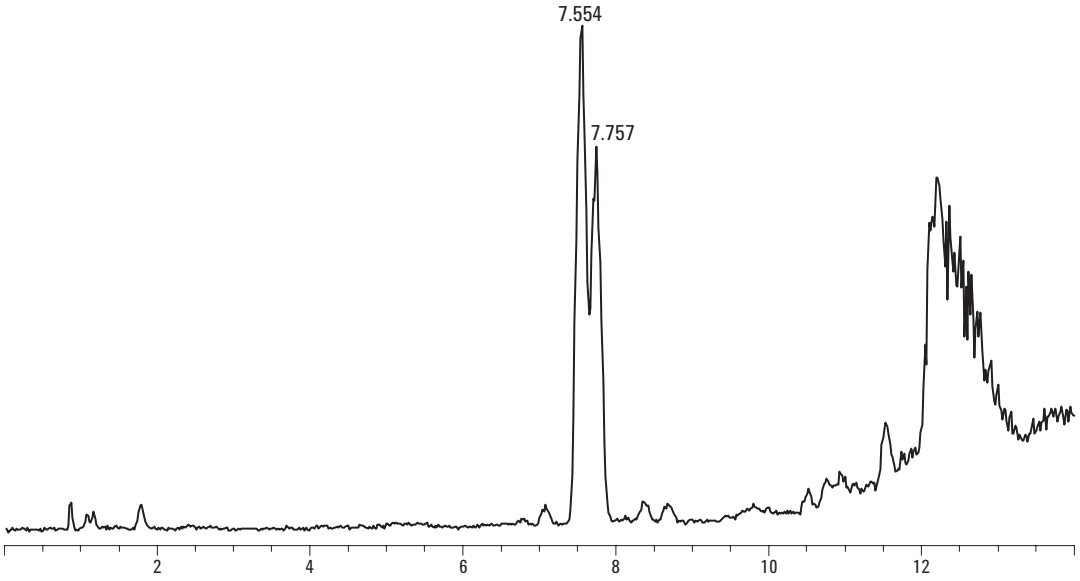

Figure 9. TIC from negative ion ESI-LC/MS of fungicide formulation.

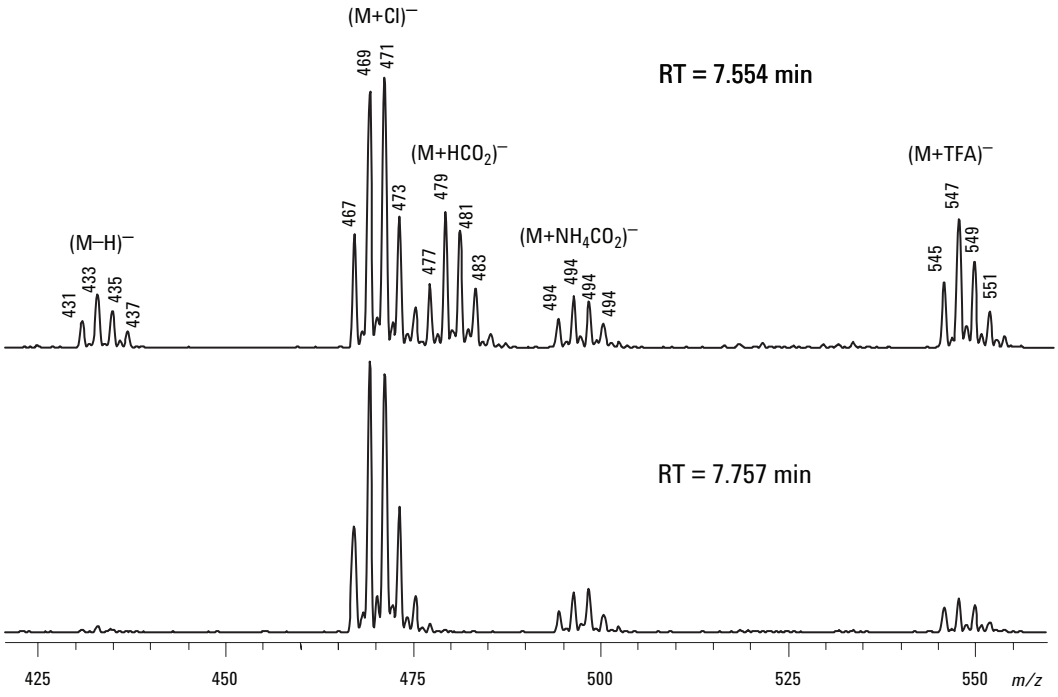

Figure 10. Negative ion electrospray mass spectra of the two triforine peaks. The spectra from peak at 7.554 minutes shows a deprotonated molecular ion (*m/z* 431) and a formate adduct (*m/z* 477) that is not seen in the later eluting peak (7.757 minutes).

Conclusions

This paper demonstrates the complimentary nature of GC/MS and LC/MS when trying to characterize a formulation that is composed of many different chemical species. The volatile compounds in the formulation can be easily separated and identified by GC/MS. In this case, polar solvents such as cyclohexanone and N-methyl-2-pyrrolidone were the major components while 1-hexanone, C9 aromatics, C10 aromatics, and substituted naphthalenes were present as minor components or contaminants. However, GC/MS did not yield any information on the active fungicidal ingredient, triforine, a hexachlorinated compound. This was most likely due to thermal decomposition during GC/MS analysis. Evidence for this was seen in a broad chromatographic hump containing chlorine-containing constituents.

The nonvolatile components in this fungicide were quickly analyzed by ESI-LC/MS. This analysis yielded information on several polar contaminants, some containing chlorine, which may be by-products of triforine production or triforine breakdown products. Also observed were several surfactants that are used in agricultural products as dispersants. The LC/MS analysis did yield significant information on the triforine active ingredient, showing a distribution of stereoisomers in the formulation.

References

1. Willoughby, R., Sheehan, E, and Mitrovich, S. A., Global View of LC/MS: How to Solve your Most Challenging Problems, p. 80, Global View Publishing, 1998.
2. The Pesticide Manual 9th Edition, Worthing, C.R. and Hance R.J. eds., p. 853, The British Crop Protection Council, 1991.

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